Matrix-Assisted Laser Desorption/Ionization Fourier Transform Mass Spectrometry of Luteinizing Hormone Releasing Hormone–Metal Ion Complexes

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Complexes of luteinizing hormone releasing hormone (LHRH) with divalent metal ions (Ni, Zn, Cu) have been studied by matrix-assisted laser desorption ionization (MALDI) and Fourier transform mass spectrometry. LHRH–metal complexes were detected in high abundance for all three metals from synthesized samples, particularly in negative ion mode. The mixture of the apopeptide with the metal salts yielded in most cases a very minor signal of metal–complex ions. As opposed to Ni and Zn, copper complex ions were mostly observed as Cu(I) adducts. This can be partly attributed to plume reactions of Cu(I) with the apopeptide. the Cu(II) complexes appeared only for the synthetic complex. We show how to distinguish between the contribution to the overall signal from desorbed complexes and from Cu(I) complexes formed in the MALDI plume. (J Am Soc Mass Spectrom 1999, 10, 19–26) © 1999 American Society for Mass Spectrometry

Transition metal ions are known to affect a number of biological processes by altering peptide or protein structures. The binding of metal ions reduces the number of accessible conformations and, hence, the conformational entropy. Metal binding is thus very effective for inducing secondary structures in smaller proteins [1].

Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are soft ionization techniques which are typically used for peptide and protein analysis by mass spectrometry. ESI has gained wide acceptance as a tool for the analysis of noncovalent complexes [2]. The observation of coordinate-covalent and noncovalent binding complexes has been far more elusive by MALDI-MS and work is needed on model compounds in order to get more information about the reliability of the technique for such investigations [3].

Luliberin (luteinizing hormone releasing hormone, LHRH) is a decapeptide amide of the sequence pGlu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2. The best known action of this brain hormone is the induction of luteinizing hormone (LH) and follicle stimulation hormone (FSH) release from the hypophysis. In vivo studies have shown that the metal complexes of the hormone have different biological activities than the free hormone. For instance, metal complexes of LHRH inhibit ovulation, but increase the release of LH and FSH [4, 5]. LHRH was chosen as a model compound because of its affinity for Cu(II), Ni(II), and Zn(II) metal ions in solution has been measured and because there are reliable methods to synthesize the metal complexes of the peptide.

Gerega et al. [6] have studied the complexation of Cu(II) and Ni(II) with LHRH in solution by means of electron spin resonance, ultraviolet spectroscopy, circular dichroism, and potentiometry. They have demonstrated that the primary binding site for the metal ion was the imidazole nitrogen (N_{im}) of the histidine residue. In solution at pH between 5.5 and 9.5, Cu forms a 3N complex by simultaneous deprotonation of two amide nitrogens: the NH from the amide bond between His and pGlu, and the NH from the pGlu. Ni forms mainly a 1N complex between pH 5 and 7.5 with a coordination to the N_{im} of the His (and possibly to carbonyl oxygens). At higher pH, Ni promotes deprotonation of up to three amide nitrogens towards the C terminal. Bal et al. [7] have studied the complexation behavior of LHRH with Zn in solution by potentiometric methods and NMR. The complexes formed are less stable than for Cu and Ni, and no deprotonation of amide nitrogens occur. The Zn is coordinated to the N_{im} of the His residue and to the carbonyl oxygen of the His–Trp bond. From these studies, the affinity of LHRH for the metal ions in aqueous solution follows the order Cu > Ni > Zn.

Another interesting feature of this peptide is that its

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N terminal is blocked (pyroglutamic acid) and its C terminal amidated, thus reducing its ability to react with divalent metal ions in the gas phase [8].

In MALDI, the analysis of metal ion complexes can be complicated by the appearance of artifactual adduct species originating from ion–molecule reactions in the MALDI plume. In most MALDI studies of metal–peptide complexation reported so far, samples were prepared by mixing the apopeptide (or apoprotein) solution with a solution of the metal salt. In many cases, in order to displace the association equilibrium, a large excess of metal was used [9]. However, even if some intact peptide–metal complex precipitates, the high salt content of the samples deteriorates the MALDI signal and may favor cationization in the plume rather than direct desorption of the complex.

Some authors have suggested the use of nonacidic matrices in order to avoid dissociation of the metal–peptide complexes during sample preparation [10]. Using this approach, they have been able to observe both noncovalent metal-binding and peptide–enzyme complexes. Such matrices were also used for probing other noncovalent associations by MALDI and yielded good results [11, 12].

In the present work, we compare the MALDI spectra of synthetic Cu, Ni, and Zn complexes of LHRH and of mixtures between the peptide and metal salts. If plume reactions were the major source of the metal-containing ions observed, the two methods should give identical results because the metal:peptide ratio was kept constant in all experiments. However, as detailed below, the two approaches gave very different results. Analyses of a mixture of LHRH with a silver salt, as well as competition experiments were also carried out, in order to further distinguish nonspecific adduct formation in the MALDI plume from the desorption of preformed complex ions.

For the present study, a mass resolution greater than 2000 at $m/z \approx 1300$ was required to resolve the isotopic patterns. Although this resolution can be attained with new generation time-of-flight (TOF) instruments equipped with delayed extraction and reflectrons, it is out of reach with classical TOF instruments. Fourier transform mass spectrometry (FTMS) is very well suited for such studies because a resolution of 10,000 or above is routinely achieved. Moreover, good mass precision, another feature of FTMS, is necessary for unambiguous determination of the oxidation state of the metal in the complex ions.

Experimental

Materials

LHRH was obtained from Sigma (Buchs, Switzerland) and used without purification. Paranitroaniline (PNA) was also obtained from Sigma. It was recrystallized from hot ethanol solution by slow cooling after addition of boiling water. Metal salts $Cu(NO_3)_2$, $CuCl_2$, $Cu(CH_3COO)_2$, $NiCl_2$, and $ZnCl_2$ were purchased from Fluka (Buchs, Switzerland); CF_3COOAg and $Ni(NO_3)_2$ were obtained from Aldrich (Buchs, Switzerland).

Synthesis of LHRH–Metal Complexes

LHRH–metal complexes were synthesized according to published procedures [4]. LHRH–Ni(II) and LHRH–Zn(II) complexes were obtained by mixing an ethanol solution of LHRH with freshly precipitated $Ni(OH)_2$ or $Zn(OH)_2$. LHRH–Cu(II) was prepared by fusion of ethanol solutions of the peptide and of copper dodecanoate. The ethanol was vacuum evaporated, and the residue was dissolved in water, extracted with chloroform, and the aqueous layer vacuum evaporated. The copper dodecanoate was prepared from copper acetate according to the procedure described by Seddon et al. [13].

MALDI Sample Preparations

Two different preparations were used throughout this work. In the first set of experiments, the metal complexes of LHRH were prepared either as suspensions (Ni and Zn, $\sim 10^{-6}$ mol of complex per mL of solvent after sonication) or solutions (Cu, 10^{-3} M) in ethanol. 5 μL were mixed with 3 μL of a PNA solution in ethanol (10 mg/mL), deposited on the probe tip, and air dried. In the other set of experiments, 5 μL of an apopeptide solution in water (10^{-3} M) was mixed with 5 μL of an aqueous metal salt solution (0.01 M). 6 μL of an ethanolic PNA solution (10 mg/mL) were added, 10 μL of the final mixture were deposited on the probe tip, and the sample was air dried. Using the latter procedure, the metal and the peptide should associate to some extent in the solution. The presence of the apopeptide and free metal is however very likely. The peptide-to-metal molar ratio was always set to 1:1. The laser pulse energy was adjusted just above threshold for ion production (estimated irradiance on the sample between 2 and 5×10^6 W cm^{-2}).

Mass Spectrometry

All experiments were performed on a homemade FTMS with an elongated cylindrical cell. the instrument is equipped with a 4.7 tesla magnet (Bruker, Bremen, Germany) and Odyssey acquisition electronics (Finnigan, Madison, WI). A Nd:YAG laster operated at 355 nm (Minilite ML-10, Continuum, Santa Clara, CA) whose beam was collinear with the axis of the cell (laser incidence angle = 90°) was used for desorption / ionization. The energy on the sample was controlled via the variation of the delay between the flash lamps and the Q-switch. The maximum output energy of the laser is 4 mJ at a Q-switch delay of 200 μs. Following ionization, the ions were allowed to drift inside the trapping cell by using the gated trapping technique (0 $\rightarrow$ 9 V). After 120 μs, the trapping voltage was set to its

Table 1. Summary of the principal species observed for the MALDI/FTMS of LHRH, LHRH–metal complexes, and mixtures of LHRH with metal salts in PNA. M = LHRH, R = CH_2O. The [y_8 + Ni] signal has a relative intensity of only 2.5%

	Positive ions			Negative ions		
Sample	Base peak	Most abundant metal containing species	Other species	Base peak	Most abundant metal containing species	Other species
LHRH	[M + H]			[M − H − R]		[M − H]
LHRH–Cu complex	[M + Cu]	[M + Cu]	[M + H] [M + Na]	[M − 2H + Cu] [M + Cu − H + Na]	[M − 2H + Cu(I)] [M − 3H + Cu(II)]	[M − H − R] [M − 2H + Cu(I) − R] [M − 3H + 2Cu(I)] [M − 4H + Cu(II) + Cu(I)]
LHRH and $CuCl_2$	[M + H]	[M + Cu]		[M − H − R]	[M − 2H + Cu(I)]	[M − H] [M − 2H + Cu(I) − R]
LHRH–Ni complex	[M + H]	[y_8 + Ni]	[M − H + Ni]	[M − 3H + Ni]	[M − 3H + Ni]	[M − H − R] [M − 5H + 2Ni]
LHRH and $NiCl_2$	[M + H]	[y_8 + Ni](trace)		[M − H − R]	[M − 3H + Ni]	[M − H]
LHRH–Zn complex	[M + H]	[z_7 − 3H + Zn]	[M − H + Zn]	[M − 3H + Zn]	[M − 3H + Zn]	[M − H − R] [M − 5H + 2Zn]
LHRH and $ZnCl_2$	[M + H]	Not detected		[M − H − R]	[M − 3H + Zn]	[M − H]

nominal value (±1 V). A 100-ms delay followed, after which the ions were excited by a radio-frequency chirp. Detection was then accomplished at an acquisition rate of 400 kHz using 128 KB of memory. The resulting transient was apodized (Blackman–Harris 3 terms [14]) and zero filled twice before Fourier transform to produce a mass spectrum.

Results and Discussion

For each metal ion (Cu, Zn, and Ni), we compared the MALDI mass spectra of the synthesized complex (LHRH–metal) and of the mixture of the metal salt with the apopeptide. PNA was chosen as a matrix because of its low acidity in order to preserve the complexes [10–12, 15]. Similar experiments done with 2,5-dihydroxybenzoic acid (pK_a = 2.97) failed to yield complex ions in most cases.

Table 1 summarizes the results obtained in positive and negative ion mode. The observed signals support the hypothesis that direct desorption of preformed species occurs to a significant extent in the case of the synthesized complexes. The results obtained for Ni and Zn are very similar and they differ from those obtained for Cu. Therefore, we will discuss copper separately.

Experiments with Ni and Zn

LHRH–Ni and Zn complexes Figure 1 shows the positive and negative ion spectra of the LHRH–Ni complex. These results are also representative for the synthesized LHRH–Zn complex.

In positive mode (Figure 1a), the main ion detected is the protonated peptide $[M + H]^+$. As the complex should be deposited intact on the probe and there should be little or no free peptide nor free metal remaining, this species must result from the fragmentation of the complex. It is obvious that in positive ion

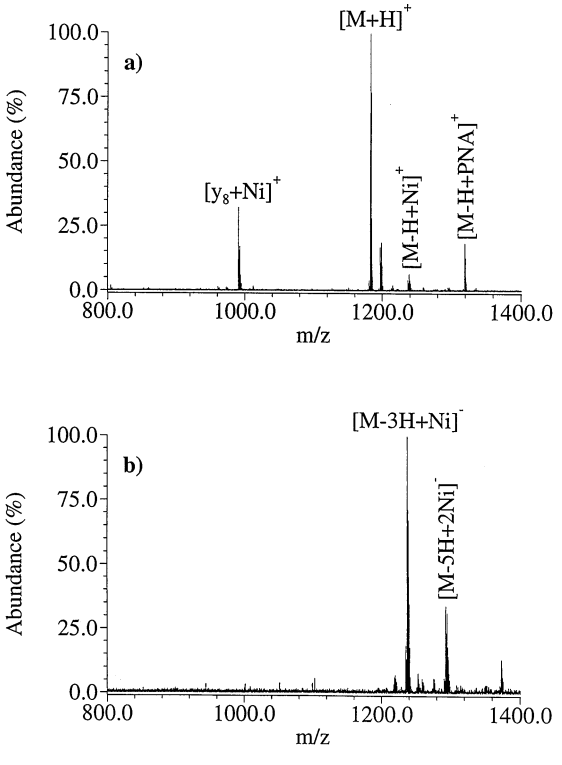

Figure 1. MALDI FTMS spectra of the LHRH–Ni complex in positive (**a**) and negative (**b**) ion modes.

mode, the Ni and Zn complexes of LHRH fragment quite extensively during the MALDI process. The peaks corresponding to the complexed LHRH $[M - H + Ni(II)]^+$ or $[M - H + Zn(II)]^+$ are observed with low abundance. For both metals, a fragment which contains one metal atom was detected. For the Ni complex, this fragment was observed at m/z 990 and was assigned to $[y_8 + Ni]^+$. This fragment corresponds to the loss of the N terminal pGlu–His fragment of the peptide. It can be related to the classical C-terminal $[y_n + 2H]^+$ fragments which typically arise from protonated peptides even though this was not observed in the case of the apopeptide (data not shown). The Ni ion is known to be primarily coordinated to the imidazole nitrogen of the histidine [6] and thus is losing its primary coordination site upon formation of $[y_8 + Ni]^+$. The conservation of the metal on the fragment can be explained by the fact that Ni can also coordinate to deprotonated amide nitrogens closer to the C terminal. In the case of Zn, a different fragment appeared at m/z 792 which was tentatively attributed to $[z_7 - 3H + Zn]^+$. This is more unusual fragmentation and we have not been able to find a rational explanation for its occurrence.

In negative mode (Figure 1b), ions corresponding to $[M - 3H + Ni(II)]^-$ and $[M - 3H + Zn(II)]^-$ are detected as the base peak. The deprotonated peptide and the fragment at m/z 1150 $[M - H - CH_2O]^-$ showed up with much lower intensity. The presence of peaks containing two metal atoms can results either from gas phase reactions, or from the desorption and subsequent fragmentation of higher order aggregates present in the solid sample.

The higher relative abundance of anions containing the nickel, compared to the analogous cationic signals, can be explained by the intrinsic gas-phase acidity of the amide NH which is similar to that of the carboxylic acid OH [16]. Therefore, as Ni favors the deprotonation of the amide nitrogen in solution, it must proceed easily with the desorption of the complex. In the case of Zn however, the deprotonation of amide nitrogen does not occur in solution [6]. Based on our observation of a triply deprotonated LHRH–Zn complex, we conclude that this process can occur in the gas phase.

Negatively charged peptide metal complexes have been observed by other groups by fast atom bombardment (FAB) of alkaline earth or transition metal ions and tripeptides [8, 17, 18]. These authors stated that the negative ions which are preformed in solution may be easily desorbed. Moreover, they reported that whereas alkaline earth metal ions in solution do not form complexes in which the amide NH groups are deprotonated, they produce triply deprotonated anions by FAB [8]. We observed the same behavior for LHRH–Zn complex anions by MALDI. These anions are expected to be observed with high relative intensity compared to their positive counterparts because the deprotonation of amide nitrogens allows an increase in the coordination number of the metal.

Whereas many differences between positive and negative ion data can be attributed to stability, at least some may be under kinetic control. In addition, as evident from the signal-to-noise ratios, the relative intensities of anionic complexes are generally lower than those of the cationic species. This implies that in some instances the anionic complexes may be more stable than other possible anions (thus dominating negative ion spectra) while still being less stable (and/or less abundant) than the corresponding cations on an absolute scale.

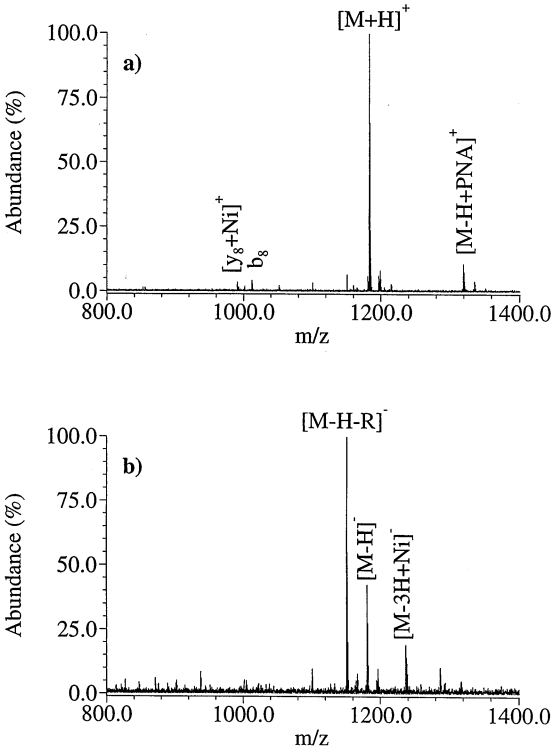

Figure 2. MALDI FTMS spectra of a mixture of LHRH and $NiCl_2$ in positive (**a**) and negative (**b**) ion modes. R = CH_2O.

Mixture of metal salt and peptide When a mixture of LHRH with $NiCl_2$ or $ZnCl_2$ in PNA was applied to the probe tip, the protonated peptide ion was the major species observed in positive mode, whereas its fragment $[M - H - CH_2O]^-$ was the main detected negative ion (see Figure 2). A minor signal arising from metal complexes, in both positive and negative ion modes, was detected. this signal was always much lower than the ones from the apopeptide-related species even when a 10-fold molar excess of metal salt was added.

The detected complex ions may originate from direct desorption of "preformed complexes" or from associations in the plume. The peptide-to-metal ratio was kept the same (1:1) in the experiments with the mixture and with the synthetic complex, thus the contributions from plume reactions should be very similar or identical. If we suppose that in the case of the mixture, all complex ions are formed via plume reactions, we must consider

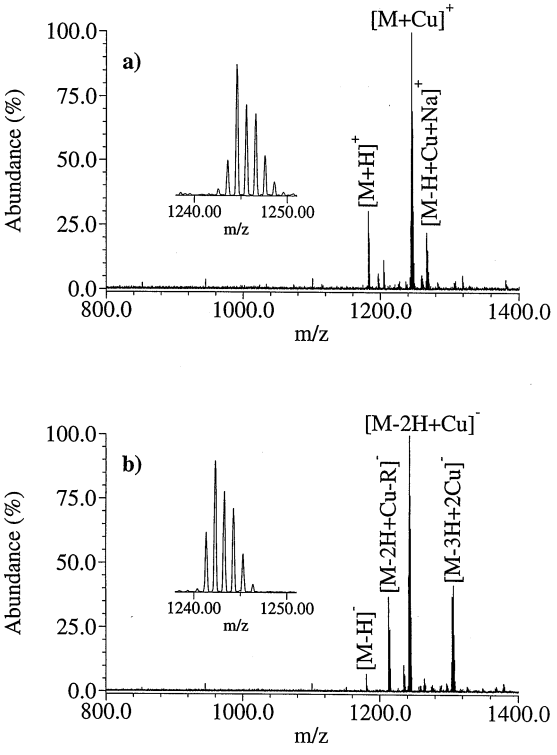

Figure 3. MALDI FTMS spectra of the LHRH–Cu complex in positive (**a**) and negative (**b**) ion modes (insets: isotopic distributions). R = CH_2O.

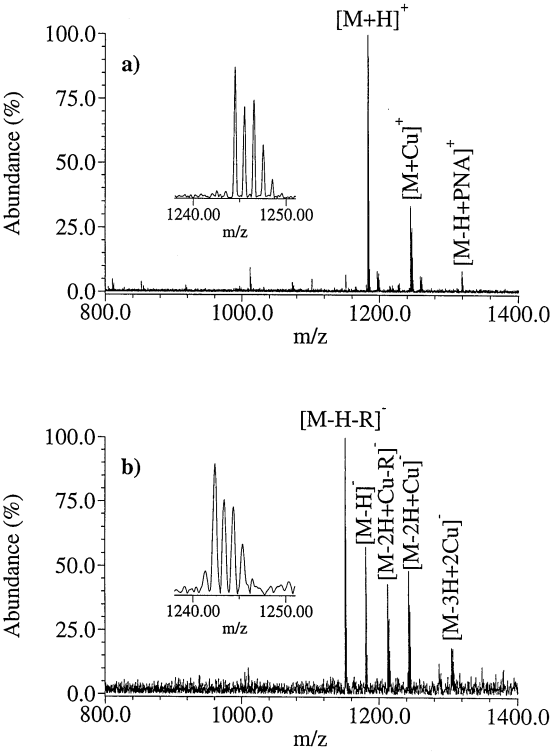

Figure 4. MALDI FTMS spectra of a mixture of LHRH and $CuCl_2$ in positive (**a**) and negative (**b**) ion modes. No Cu(II) complexes are evident from the isotopic patterns (insets). R = CH_2O.

another origin for the much higher intensities observed for the synthesized complex. Thus, at least the difference in abundance of the LHRH–metal ions between both experiments can be attributed to the desorption of intact complexes.

Experiments with Cu

Figure 3 shows the spectra obtained from the LHRH–Cu complex and Figure 4 the spectra from a mixture of the apopeptide with $CuCl_2$. In positive ion mode the copper-containing species $[M + Cu]^+$ was detected for both samples. It was much more abundant for the synthetic complex (Figure 3a) than for the mixture (Figure 4a) where $[M + H]^+$ was the major ion detected. In negative mode the same kind of behavior was observed. The $[M - 2H + Cu]^-$ species was also detected in both cases, but with much higher relative intensity in the case of the synthesized complex (compare Figures 3b and 4b).

As we have argued in the case of Ni and Zn, at least the difference in intensity of the complex ion in both experiments can be attributed to the desorption of preformed ions. For copper, the contribution of plume reactions is nevertheless more significant than for the two other metals, despite the greater stability of the Cu(II)–LHRH complex in solution. The peptide–metal salt mixtures were prepared with $Cu(II)Cl_2$. As opposed to Ni and Zn, copper mainly appeared as Cu(I) in both samples and in both polarities, i.e., $[M + Cu(I)]^+$ and $[M - 2H + Cu(I)]^-$. The reduction of copper from Cu(II) to Cu(I) has already been observed in other MALDI experiments [19]. These authors stated that the origin of the Cu(I) ion is unknown, but it was tentatively attributed to the photochemical reduction of Cu(II).

In the case of the synthesized LHRH–Cu complex, the isotopic distribution of the peak assigned as $[M + Cu]^+$ showed a Cu(II) contribution besides Cu(I). Figure 5a, b show the calculated isotopic distributions which should be observed from $[M - H + Cu(II)]^+$ and $[M + Cu(I)]^+$, respectively. Figure 5c shows the theoretical pattern for 25% of Cu(II) and 75% Cu(I), along with the observed spectrum. A Cu(II) contribution is detected in negative mode as well. The $[M - 3H + Cu(II)]^-$ ion accounts for approximately 40% of the total complex intensity. Cu(II)-containing ions were hardly detected in the case of the mixture (see insets Figure 4). Most of the copper must therefore be reduced during the MALDI process. It is, however, unclear whether the copper is reduced prior to reacting with the apopeptide in the plume, or if the desorbed Cu(II) complex undergoes a reduction by capturing a hydrogen atom. The very high abundance of the Cu(I)-containing species in the case of the synthesized complex compared to the mixture suggests a reduction of the desorbed complex.

In the negative ion spectrum of the synthetic complex, higher order adducts of Cu(I) to the complex are detected. $[M - 4H + Cu(II) + Cu(I)]^+$ and $[M -$

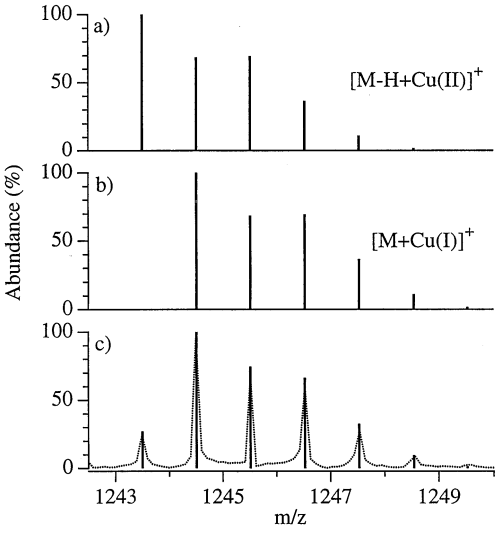

Figure 5. Isotopic distributions expected for (**a**) pure $[M - H + Cu(II)]^+$, (**b**) pure $[M + Cu(I)]^+$, and (**c**) a 25:75 mixture of both species with, overlaid, the experimentally observed distribution of the synthetic LHRH–Cu complex.

$3H + 2Cu(I)]^-$ whose isotopic distributions overlap are particularly intense. The fact that higher order adducts are formed by addition of Cu(I) only [and not Cu(II)] suggests that Cu(I) is the reactive species in the plume and that the Cu(II) complex is likely to be desorbed intact from the solid phase. The same behavior has previously been observed in the case of the adrenocorticotropic fragment 4–11 [20].

Isotopic patterns resulting from Ni and Zn were also inspected. These did correctly fit the theoretical distributions, and there was no evidence for more than one oxidation state.

Experiments with Ag(I)

Mixture of the peptide with a silver salt. In order to further distinguish the addition of Cu(I) in the plume from the desorption and subsequent reduction of preformed LHRH–Cu(II) complexes, we performed control experiments in which the copper salt was replaced by a silver salt. Ag(I) was chosen because, like Cu(I), it has a d^{10} electron shell and its behavior should be very similar to that of Cu(I), the main difference being their ionic radii [21]. Moreover, unlike copper, silver is not expected to from stable complexes with the peptide in solution. Ag adducts to the peptide should thus arise only from plume reactions.

When Ag(I) is mixed with the apopeptide, the $[M + Ag]^+$ ion is detected in the positive ion spectrum (see Figure 6a). In negative ion mode, the Ag adduct to the peptide $[M - 2H + Ag]^-$ is detected with low relative abundance together with its fragment at m/z 1257 which results from the loss of the serine side chain (CH_2O) (see Figure 6b). These spectra show the same features as those obtained from the mixture with a copper(II) salt (compare

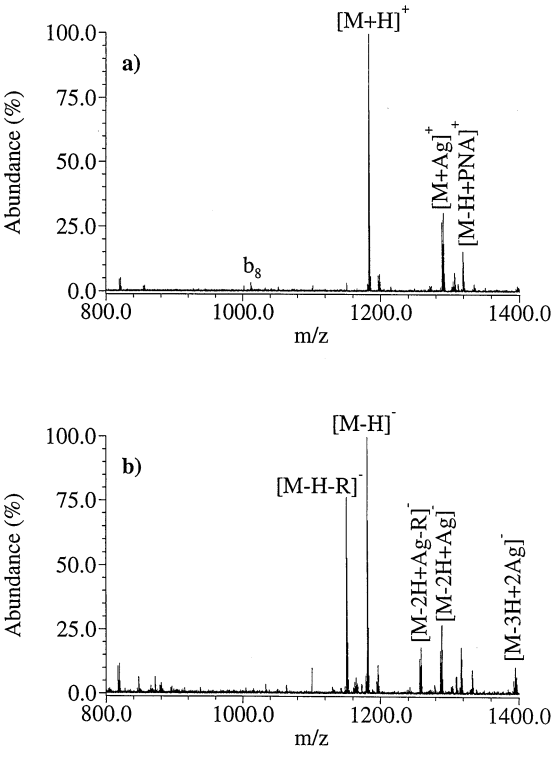

Figure 6. MALDI FTMS spectra of a mixture of LHRH and CF_3COOAg recorded in positive (**a**) and negative (**b**) ion modes. R = CH_2O.

Figures 4 and 6). This lends additional support to the interpretation that the copper-containing species ($[M + Cu]^+$, $[M - 2H + Cu]^-$) detected for the LHRH–Cu and the LHRH mixture with a copper salt originate from plume reactions of Cu(I) with the peptide.

In the case of copper, we have seen that a very high signal of $[M + Cu]^+$ and $[M - 2H + Cu]^-$ is detected upon laser desorption of samples prepared with the synthetic metal complex. This signal is much more intense than the one detected for the mixture. The same observation holds if we compare the $[M + Ag]^+$ signal of the mixture with the silver salts (Figure 6) and the $[M + Cu]^+$ signal from the synthesized complex (Figure 3). To account for that, we must assume that not only Cu(II)-containing species are formed by direct desorption, but that some Cu(II) complex ions are subsequently reduced in the MALDI plume, e.g., by capturing a hydrogen atom left by the matrix. Hence, there is a nonnegligible contribution of preformed complex ions to the overall signal for the LHRH–Cu complex, and these ions can be detected as Cu(II) species or reduced to Cu(I) species.

Competition experiment with Ag. The similar gas-phase reactivity of Ag(I) and Cu(I), and the fact that Ag does not form a stable complex with LHRH stimulated us to consider competition experiments with the synthetic complexes. Competition between Ag(I) and Cu(II) would give very complicated spectra in which direct

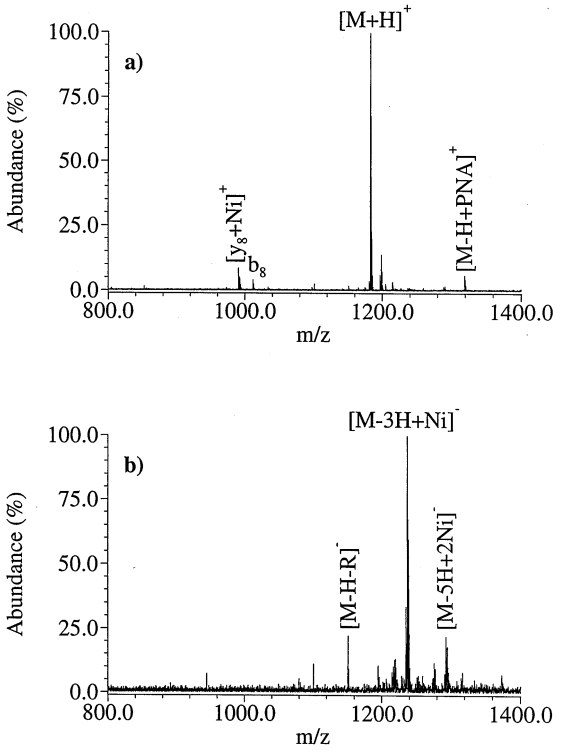

Figure 7. MALDI FTMS spectra of a mixture of LHRH–Ni complex and CF_3COOAg recorded in (**a**) and negative (**b**) ion modes. R = CH_2O.

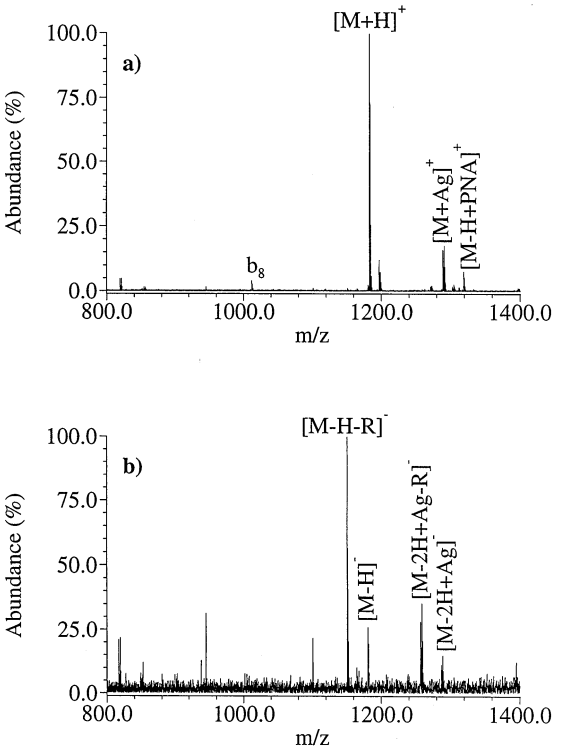

Figure 8. MALDI FTMS spectra of a mixture of LHRH, $Ni(NO_3)_2$, and CF_3COOAg in a 1:1:1 molar ratio in positive (**a**) and negative (**b**) ion modes. R = CH_2O.

absorption of Cu complexes as well as cationization by Cu(I) or Ag(I) in the plume all contribute to the detected species. Hence, the competition experiments were carried out with silver and nickel because we have shown that the contribution to the complex ion signal from plume reactions with Ni(II) is very weak compared to the intact desorption of complex. In the following experiments, Ni complexes result from intact desorption, whereas Ag adducts result from plume reactions.

Figure 7a, b show the positive and negative ion spectra obtained from a mixture of the LHRH–Ni complex and 1 molar equivalent of CF_3COOAg in PNA. We have seen above that when Ag(I) is present with the apopeptide, it reacts to form an abundant $[M + Ag]^+$ peak in positive ion mode (see Figure 6a). For the mixture of the silver salt with the LHRH–Ni complex, however, the $[M + Ag]^+$ peak is hardly detected, and the spectrum is dominated by the $[M + H]^+$ ion and the LHRH–Ni complex fragment at $[y_8 + Ni]^+$ (see Figure 7a). This shows that the $[M + H]^+$ ion does not originate from protonation of the free peptide because it would also react with the silver. The complex is thus shown to survive the deposition in the matrix solid phase. In addition, this demonstrates that the $[M + H]^+$ peak is indeed a fragment from the metal complex. In negative ion mode, the same kind of results are obtained, and the spectrum looks very much like the one obtained without the silver salt (compare Figures 6b and 7b). This confirms that in the case of the Ni–LHRH complex, the contribution to the $[M - H + Ni]^+$ signal from plume reactions is almost negligible.

When the sample is prepared by mixing the apopeptide with a solution containing both $Ni(NO_3)_2$ and CF_3COOAg in proportion 1:1:1 prior to adding the matrix, the spectra (see Figure 8) are very different than the ones obtained for the synthetic complex. In positive ion mode, a peak of Ag cationized peptide appears together with an intense protonated peptide ion. In the negative ion mode, the major species detected are $[M - H - CH_2O]^-$, $[M - H]^-$, and Ag adducts to the peptide. These spectra look very similar to those obtained when mixing the apopeptide with only the silver salt. We observe that for the mixture of the peptide with the two salts, the free peptide is desorbed and reacts with the silver in the plume. The fact that no Ni complexes are detected confirms that those which are observed for the synthetic complex are not formed in the plume but must emerge from the solid phase during the laser desorption process.

Our interpretation of these findings is that the divalent metal complexes of LHRH preferably form negative ions by multiple deprotonation of the peptide chain, presumably at amide nitrogens. This in turn produces a large amount of protons which can attack the metal binding site of other LHRH molecules, disrupting the complex and causing the $[M + H]^+$ species to be so abundant in positive mode.

Conclusion

This work using LHRH–metal complexes as model compounds clearly shows that metal complexes of biomolecules can survive the MALDI sample preparation and the laser desorption/ionization step if a nonacidic matrix is used. In the case of Cu, a significant contribution from plume reactions was found, but for Ni and Zn, we have shown that under the conditions used throughout this work, there is negligible cationization in the plume. The plume reactions in the case of copper can be attributed to the addition of Cu(I) to the free peptide. Moreover, desorption of LHRH–Cu(II) complexes and subsequent reduction to LHRH–Cu(I) must also occur to account for the very intense signal of the $[M + Cu(I)]^+$ and $[M - 2H + Cu(I)]^-$ signals detected in the case of the synthetic complex.

We have shown as well that negative ion spectra are very useful because the complex anions were found to be of high relative abundance. This is probably the result of the high acidity of the amide NH groups of the complex in the gas phase. In the case of mixtures of the apopeptide with metal ion salts, intact complex ions are hardly detected in most cases.

It is important to stress that in order to be detected, the complex must be present in the sample at high concentrations compared to the apopeptide. Otherwise, the risk of detecting nonspecific adducts is very high. Thus, the mixture of metal salt with peptides does not always represent a reliable procedure for the preparation of samples for the MALDI analysis of metal–peptide complexes. A reason for this may be the complexing ability of the matrix itself [22] which can prevent the formation of the peptide complex.

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